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Brief Communication

Evidence for the Colonization of Lactic Acid Bacteria in the Gastrointestinal Tract of Suckling Mink

Lactic acid bacteria are considered indigenous members of the gastrointestinal microflora in a number of animal species *(Savage* 1977a). Some intestinal strains of lactobacilli and streptococci are able to adhere to stratified squamous epithelium of some animals *(Tannock et al.* 1987), in the non-secreting part of the stomach of piglets *(Barrow et al.* 1980, *Fuller et al.* 1978) and rodents *(Tannock et al.* 1982), and in the crop of poultry *(Fuller* 1978). The presence of lactic acid bacteria in the digestive tract is believed to be of beneficial value to the host animal *(Fuller* 1989). The production of organic acids in the stomach or the crop helps maintaining a low pH which may be important for inhibiting the colonization of potentially pathogenic bacteria, particularly in the newborn animal *(Barrow et al.* 1980, *Fuller* 1977, *Fuller* 1978). The adhesion of lactobacilli to squamous epithelium is host specific: strains capable of adhering to the epithelium of piglets are usually not able to adhere in rodents or poultry and vice versa *(Fuller* 1978, *Lin & Savage* 1984, *Tannock et al.* 1982). Adhesion of lactic acid bacterial strains to other epithelia than stratified squamous epithelium has been reported. Thus, the attachment of lactobacilli to cells from the secreting epithelium of the murine stomach *(Kotarski & Savage* 1979), to intestinal cells of humans *(Goldin & Gorbach* 1987), and to columnar epithelial cells of piglets and calves *(Mäyrä-Mäkinen et al.* 1983) has been demonstrated using in vitro methods. In another study the in vivo attachment of *Enterococcus faecium* to duodenal epithelium of gnotobiotic chickens was demonstrated *(Fuller et al.* 1981). Recent research indicated that in adult mink lactic acid bacteria are not indigenous members of the intestinal flora, and they do not attach to epithelium in any part of the gastrointestinal tract *(Pedersen & Jørgensen* 1992). The present paper presents evidence that Gram positive cocci may colonize the gut of suckling mink kits and attach to the gut mucosa.

In order to study the intestinal flora of suckling mink kits, 10 few days old kits from 10 different litters were killed by intracardiac or intrathoracic injection of pentobarbital sodium for bacteriological examination and scanning electron microscopy.

Samples from the oesophagus, stomach, and jejunum were immediately collected for scanning electron microscopy and kept in a fixative of 3% glutaraldehyde, 0.1 M cacodylate, and 0.15 M calcium chloride, pH 7.2, until further processing. Specimens were then trimmed and subsequently dehydrated through a series of baths of increasing concentrations of acetone (25%, 40%, 60%, 90%, 100%, 100%, 100%), critical point dried, mounted on metal stumps, and coated with gold as previously described *(Pedersen & Tan-*

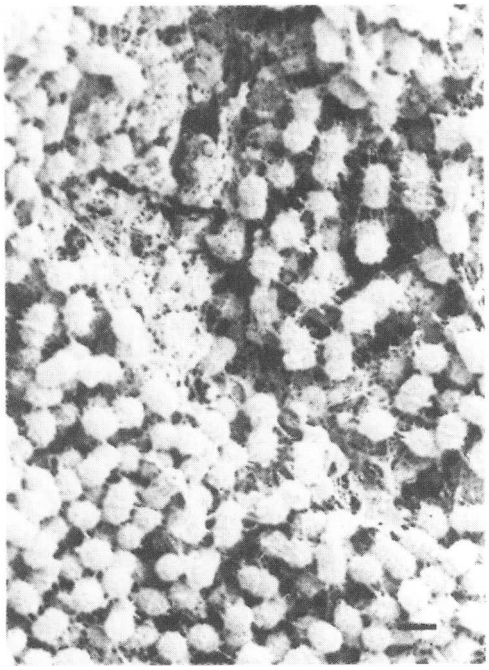

Figure 1. Scanning electron micrograph from the jejunum of a suckling mink kit. Numerous cocci are present in the jejunal contents. Magnification×6000. Bar = 1 μm.

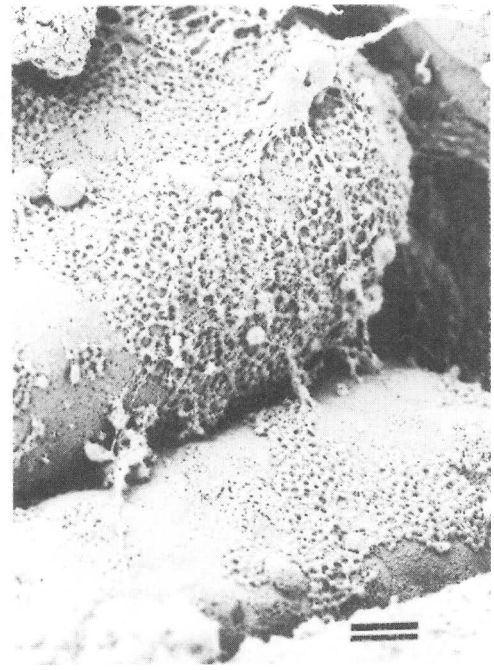

Figure 2. Scanning electron micrograph showing villi from the jejunum of a suckling mink kit. A patchy layer of cocci covers parts of the mucosa. Magnification×1000. Bar = 10 μm.

nock 1989). Scanning was performed in a JEOL JSM 840 A scanner.

For bacteriological examination, the stomach together with sections, approximately 2 cm long from the oesophagus, and 5 cm long from the jejunum were excised and flushed through with sterile saline (0.9% NaCl w/vol) according to the protocol of *Pedersen & Tannock* 1989. The saline solution was recollected after washing through the intestine and diluted in serial 10-fold dilutions in sterile saline before plating on agar media. Lactobacilli were

Table 1. Counts of streptococci/enterococci/lactococci, *E. coli*, lactobacilli, staphylococci and clostridia in the oesophagus, ventricle and jejunum of suckling mink kits. Counts are expressed as cfu per ml.

Bacteria	Oesophagus	Ventricle	Jejunum
Streptococci/enterococci/lactococci	0 - 1.7×10^4	2.6×10^3 - 8.9×10^6	1.2×10^8 - 1.8×10^8
E. coli	0	0	3.0×10^2 - 2.1×10^4
Lactobacilli	0	0	0
Staphylococci	0	0	1.6×10^5 - 2.0×10^5
Clostridia	0	0	0

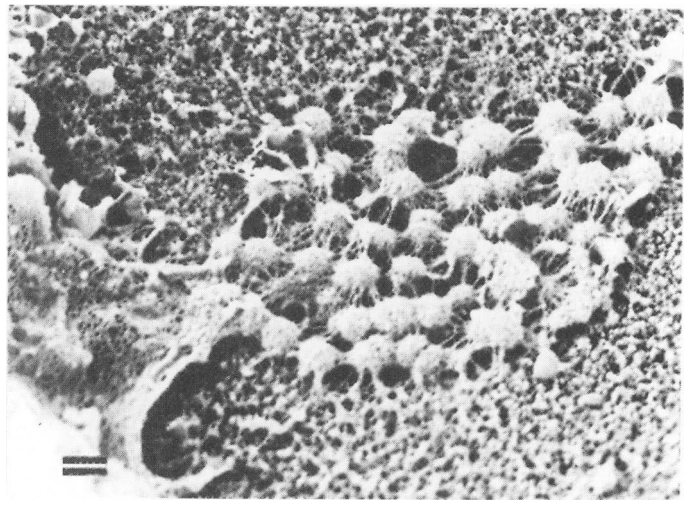

Figure 3. Close-up scanning electron micrograph showing bacteria associated with the jejunum of a suckling mink kit. To the left the bacteria are partially covered by the dehydrated mucus blanket. Filamentous structures connecting bacteria to each other and to the mucosal surface are recognized. Magnification×7000. Bar = 1 μm.

counted on Rogosa SL agar (Difco 0480-01-8), streptococci and enterococci on Mitis Salivarius agar (Difco 0298-01-0) added nalidixic acid, 15 μg/ml, instead of Chapman tellurite (*Barrow et al.* 1980), *Escherichia coli* on MacConkey agar (Difco 0075-01-9), staphylococci on Baird Parker agar (Oxoid, CM275), and clostridia on veal infusion agar added 5% bovine blood and polymyxin B, 10 i.e./ml. Lactobacilli were incubated for 48 h at 37°C in an atmosphere of 65% N_2, 25% H_2, and 10% CO_2, clostridia for 24 h at 37°C in incubation jars in an atmosphere of 70% N_2 and 30% H_2, and staphylococci and coliforms for 24 h aerobically at 37°C.

The intestinal microflora of the mink kits was very sparse. In most samples neither coliforms, staphylococci, lactobacilli nor clostridia were detected (Table 1). However, from all kits a Gram positive coccus was isolated on Rogosa SL medium from the stomach and the intestine but not from the oesophagus. In some of the kits it was present in very high numbers. Upon repeated isolation the strain was identified using an API 20 STREP kit (BioMérieux S.A., Marcy-l'Étoile, France) and it invariably came out as a *Lactococcus* species.

By scanning electron microscopy coccoid shaped bacteria were demonstrated, both in the luminal contents (Fig. 1) and associated with the intestinal mucosa (Fig. 2 and 3) of the jejunum, attached to each other and to the mucosa by a filamentous matrix. These structures, although appearing fimbriae- or fibril-like, may be artefacts due to shrinkage phenomena during the dehydration process. Similar filamentous structures have been described by other researchers and are assumed to be artefacts (*Savage* 1977b). On several specimens a layer of bacteria lying very close to the epithelium, partially covered by a dehydrated mucus blanket, could be noticed (Fig. 3). This observation may indicate that the filamentous structures are dehydrated mucus.

It was not possible from the scanning electron

micrographs to conclude whether the bacteria were attached directly to the epithelium or to components in the mucus blanket, nor was it possible to evaluate the mechanism of the attachment. Adhering bacteria were never demonstrated in the oesophagus or the stomach. This is in contrast to findings in e.g. piglets and rats (*Pedersen & Tannock* 1989, *Tannock et al.* 1987).

The natural habitats of lactococci are plant material and milk. Usually, it is not considered indigenous to the intestinal tract. Furthermore, the passage time for the food through the digestive tract of mink is very short, which makes it difficult for bacteria to colonize. The finding of this lactic acid bacterial species in such high numbers and attached to the mucosa in the jejunum of mink kits was therefore unusual.

The presence of the cocci was not associated with any clinical symptoms of disease in the kits.

Recent results suggest that lactic acid bacteria are not indigenous to the gastrointestinal tract of adult mink, and that the intestinal microflora of mink is very sparse *(Pedersen & Jørgensen* 1992). However, the addition of lactic acid bacteria to the feed of mink has been shown to significantly decrease the mortality during outbreaks of mink virus enteritis *(Jørgensen* 1991). The intestinal microbial ecology of suckling mink kits may be different from that of adult mink for nutritional reasons. The relatively high concentration of lactose in the maternel milk from mink, about 7% *(Olesen* 1987) may be of importance, since most lactic acid bacteria rapidly metabolize lactose, producing organic acids. The existence of a particular flora in the newborn animal has been shown in a number of species. The predominance of bifidobacteria in the intestine of breast-fed infants is one well documented example *(Poupard et al.* 1973). The impact of naturally aquired lactic acid bacteria on the kits is at present unknown. Further experiments should be conducted to examine the intestinal microbial ecology of mink, particularly the ecology of lactic acid bacteria.

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